22 COMPARISON OF METHODS FOR REMOVAL OF EPITHELIUM IN AIRWAYS FROM A SMALL ANIMAL. NEED FOR HISTOLOGIC VERIFICATION.

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A study on the effect of the epithelium on nicotine-induced bronchoconstriction (Lang, Hahn 1990) directed our attention to the need for a defined and histologically controlled method to remove the epithelium. Systematic histologic examination revealed the ability of some methods to cause substantial damage to the basement membrane and into subepithelial tissue. We therefore initiated a formal study of different methods for removal of epithelium in the ferret trachea.

We examined 4 techniques of epithelium removal in tracheae excised from ferrets under pentobarbital anaesthesia: (1) gentle scraping with a scapel blade moved backwards (away from the cutting edge), (2) moving a Q-tip through the unopened tracheal tube without lateral pressure, (3, 4) stroking the mucosa of opened tracheal segments with a Q-tip, exerting (3) light or (4) moderate pressure.

Method (1) removed the epithelium and most of the basement membrane, caused damage to the submucosa in most specimens and was abandoned without quantification. Each of the remaining methods was quantified in 6-8 segments by each of two observers. Since no consistent differences were noted between observers results from different observers were pooled. The table lists structures left intact by procedures (2), (3) and (4).

Method	Epithelium %, x±SE	Basement Membrane %, x±SE	Basal Cells mm^{-1}, x±SE
(2)	0.0 ± 0.0	71 ± 10	17 ± 3.0
(3)	3.1 ± 2.2	63 ± 06	13 ± 2.7
(4)	1.6 ± 0.7	41 ± 06	12 ± 3.5

Method (2) was as complete as methods (3) and (4) in removing the epithelium (efficiency of removal for all methods, 97-100%), but caused less damage to the basement membrane than the other two methods, especially method (4) which removed almost 60% of the basement membrane compared to 29% removed by method 2. In the ferret, method (2) was clearly best, followed closely by method (3). Preliminary studies on tracheae of large animals have shown a different pattern of results in that the mucosa from large animals was more resistant to removal of the epithelium. However, damage to the basement membrane was also very frequent. Complete removal of epithelium without damage to the basement membrane and/or to subepithelial connective tissue was as difficult to achieve in large as it was in small animals. We conclude that each method employed for removal of epithelium needs to be quantified histologically. Functional studies will be most difficult to interpret without such control.

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